

## Purification of Sirups from Hydrolyzed Lactose in Sweet Whey Permeate

### ABSTRACT

Sirups were prepared from whey permeates containing 90% hydrolyzed lactose by use of bentonite to remove protein and by cation and anion exchange resins to remove salts and nonprotein nitrogen, and then they were concentrated to 60 to 66% total solids. The resulting clear colorless sirups contained 98.7 to 98.4% carbohydrate, 1.18 to 1.40% protein equivalent (nitrogen  $\times$  6.38), and .13 to .23% chloride ash, dry weight. The sweetness and stability of these sirups were equal or more than sirups made by hydrolysis of purified lactose.

### INTRODUCTION

Cheese wheys are under-utilized (6) and contain high quality protein (5, 22) and large amounts of lactose which, after hydrolysis, has potential as a sweetner (7) and is a source of energy in fermentations (17). Since the consumption of cheese in the United States is increasing (6), more cheese whey is being produced. Yoghurt, cottage cheese (9), and cheddar cheese (20) have been prepared from milk containing enzymatically hydrolyzed lactose with increased rate of acid development and yield of cottage cheese, and reduced aging time for cheddar cheeses. If manufacture by these processes were implemented, significant amounts of whey containing hydrolyzed lactose (HL) would be available. Protein concentrates of nutritive value can be made from wheys by ultrafiltration (13), gel permeation (4), and electrodialysis and gel permeation. The

resulting permeates, low in protein nitrogen but high in salts and and lactose, pose a problem of utilization. Lactose can be crystallized from whey permeate, but sugar crystallization from permeates containing hydrolyzed lactose is not feasible because of its mixed composition of glucose, galactose, and oligosaccharides.

The purpose of this research was to prepare purified lactose-derived sirups from sweet whey permeates by employing ion exchange resins and protein adsorbants. This information may be useful in extending the potential utilization of HL sirups.

### MATERIALS

#### Ion Exchange Resins

Cations were removed by Amberlite IR252 (Rohm and Haas, Philadelphia, PA)<sup>2</sup> of 100 mesh in the H<sup>+</sup> form. Anions were removed by IRA 93 (Rohm and Haas), a weakly basic exchange resin, of 16 to 50 mesh in the OH<sup>-</sup> form.

#### Miscellaneous Materials

Volclay custom granular sodium bentonite (American Colloid Co., Skokie, IL) and Norit decolorizing carbon (Eastman Co., Rochester, NY) were used for removal of protein. Cane sugar was used as a standard in panel testing for sweetness. Spray-dried lactose hydrate (Foremost-McKesson, San Francisco, CA) was used to prepare hydrolyzed lactose.

#### Sweet Whey Permeate

The whey permeate was prepared by the Dairy Foods and Nutrition Laboratory at Beltsville, MD, by the addition of .32 g *Saccharomyces lactis* lactase (Maxilact)/liter to whole milk pasteurized for 15 s at 74 C. The enzyme supplied by the Enzyme Development Corp. (New York, NY) had 40,000 ONPG (ortho-nitro-phenyl-galactopyranoside) units/g. The milk was incubated 2.5 h at 31 to 32 C, then repasteurized at 74 C for 15 sec. Cheddar

cheese was prepared from the milk, and the whey was drained, pasteurized, and passed thru a pilot plant Abcor ultrafiltration unit. The permeate was then condensed to 56.4% from 5.97% total solids (TS). The analysis (dry weight) is given in Table 1; the insoluble material (probably lipoprotein), consisting of 17.5% fat, was removed by filtration of the diluted condensate before further processing.

#### Preparation of Sirups from Sweet Whey Permeate

Twelve liters of permeate diluted to 7.6% TS were treated with cationic resin and then with anionic resin. Deproteinization was with either 3.5 or 2.5 g of presoaked bentonite (1:10 water)/g calculated protein at pH 4.5 either before or after deionization. To reduce demineralization costs, deproteinized sirups were prepared by treatment of 11.7% TS permeate at pH 6.0 with bentonite, concentrated to a sirup (V), and blended with ion exchange sirups for sensory evaluation.

#### Hydrolyzed Lactose

Three liters of .584 M lactose were adjusted to pH 6.5 with .01 ionic strength  $\text{KH}_2\text{PO}_4$ -NaOH buffer, and a suspension of .1% (wt/vol) of lactase was added. After 6 h at 30 C (90% hydrolysis), the enzyme was inactivated by adjusting the pH to 4.6 with 2 N HCl. Samples then were heated to 70 C further to precipitate the protein, filtered on a suction flask with Celite filter-aid, and passed through IR 252 ( $\text{H}^+$ ). The filtrate (pH 2.03) was next passed through IRA-93 ( $\text{OH}^-$ ). After adjustment of pH from 7.0 to 5.3 with .05 ml 2 N HCl, the eluate was concentrated (in vacuo) at 65 to 70 C to a clear sirup of 66.2% TS.

TABLE 1. Analysis of dried hydrolyzed sweet whey permeate (89.4% hydrolysis).

	%
Carbohydrate	85.20
Ash	8.65
(NPN $\times$ 6.38)	2.95
(Protein N $\times$ 6.38)	1.50
Insoluble material	1.60

## ANALYTICAL METHODS

### Degree of Lactose Hydrolysis

A Leeds and Northrop Enzymax lactose/glucose analyzer was used to determine the degree of lactose hydrolysis.

### Nitrogen

Total nitrogen (TN) and nonprotein nitrogen (NPN) in the original whey permeate were determined by the Rowland method (18) by micro-Kjeldahl procedure (2). The difference (TN-NPN) equaled protein nitrogen (PN). The PN content of eluates following ion exchange was determined nephelometrically at 650  $\text{m}\mu$  in 15% wt/wt trichloroacetic acid (8) with the diluted permeate as a standard. With ash-free eluates and sirups, Kjeldahl nitrogen was determined after predigestion with 3 ml of concentrated  $\text{H}_2\text{SO}_4$  until foaming stopped before catalysts were added. In protein-free eluates or sirups prepared with excess bentonite, TN represented NPN.

### Ash

Ash was determined in whey permeate by the standard method for milk (1) and in sirups by the corn sirup method (10), in which 5 g were ashed at 525 C after addition of 3 ml of  $\text{H}_2\text{SO}_4$  (diluted with 3 vol of water).

### Color Stability

Color stability of sirups, heated to 100 C for 1 h, was determined spectrophotometrically by the optical density difference at 600  $\text{m}\mu$  and 450  $\text{m}\mu$  (11).

### Total Solids

Total solids of diluted, clarified permeates, eluates, and concentrated sirups were calculated from refractive index (RI) measurements at 27 C. As determined by the Mojonner procedure for TS (15), these RI measurements then gave a valid percentage TS for these preparations when the RI was read on the International Scale for Sucrose at 20 C. The percent sucrose equaled percent TS.

### Insoluble Solids

The percentage difference of TS for a 23% solids dilution of the permeate before and after

filtration, determined by the Mojonnier procedure (15), was used to determine percent insoluble solids.

#### Conductivity

Conductivity of permeates on eluates after ion exchange treatment was determined from the resistance of the solution at 25 C on an industrial Instruments Conducting Bridge. The measured specific conductance was calculated as the reciprocal of the specific resistance. Specific resistance equaled the resistance of the solution in ohms divided by the cell constant. A cell constant of 1.13 was determined experimentally with .010 N KCl (specific conductance  $1.41 \times 10^{-3}$  at 25 C).

#### Chemical and Biological Stability

Sirups were placed in 113 g screw cap bottles and sealed. Others were heated to 70 to 75 C for 5 min in loosely capped bottles prior to being sealed. Stability was judged as the time necessary for visible crystallization to take place or mold growth to occur.

#### Panel Evaluation

Sirups were evaluated for sweetness and other sensory attributes under subdued light by 14 trained panel members. Samples were evaluated by the magnitude-estimation procedure for sweetness in comparison with standard sucrose solutions. Each judge was supplied with a sheet marked with parallel horizontal lines. A mark placed on the extreme left signified no sweetness and a mark placed at a distance of 10 cm to the right on this line indicated the sweetness of a control. Each judge first tasted a high-solids sucrose control solution which was designated as full sweetness. After rinsing with water between samples, the judges tasted other concentrations of sucrose, a hidden control, and test sirup solutions in randomized order and estimated the sweetness by placing a mark on the line. The judges' responses were quantified by measuring the distance of these marks from the no sweetness point. The data were analyzed by computer to determine significance of the average values. Sweetness of samples were computed in terms of the sweetness of the hidden-control sucrose solutions by use of the formula  $S = kC^n$ , where

$S$  = sweetness value as a distance marked on the line,  $k$  is constant,  $C$  = concentration of sugar as percent, and  $n$  = a calculated power exponent (16).

## RESULTS AND DISCUSSION

### Purification of Lactose Hydrolysate

First attempts to purify HL whey permeates by one-pass ion-exchange procedures did not remove all of the protein and caused troublesome foaming when the eluates were condensed. Adjusting the pH of the eluates to 4.5, followed by heating at 92 C for 10 min and then filtering, only partially removed the protein. Bentonite then was used to remove protein. Bentonite has been used in the fining of wines (12) and, recently, to remove proteins from whey (3). Protein removal by bentonite adsorption is especially successful for relatively small quantities of protein. Addition of bentonite removed all the protein and 13.4% of the nonprotein nitrogen (NPN) from the sweet whey permeate (Table 2). Ion exchange resins removed additional NPN. Comparable residual N was obtained whether the protein was removed initially or after being processed through ion exchange resins. It is recommended that protein be removed initially because the protein adsorbed by the anion resin is difficult to remove during regeneration. All final eluates were adjusted to pH 5.0 to 5.3 with either 2 N HCl or 2 N NaOH.

Norit decolorizing carbon (1%) added to a 7.6% total solids (TS) permeate adsorbed 24% protein and 12.8% NPN. Combining bentonite and Norit treatments reduced NPN still further and removed all protein (Table 3). The conductivity of the treated permeate was increased slightly by adjustment of the pH to 4.5. The eluate was passed through the cation resin and then through the anion exchange resin. Since eluate M contained protein leached from the column, the bulk of the eluates (K, L, and P) were combined and passed again through the anion resin. This second passage did not change the NPN value from that of the combined calculated weighted value of the eluates passed through the ion exchange resins the first time or that of the material passed through the cation resin.

TABLE 2. Analysis of sweet whey permeate after treatment with anion and cation exchange resins.

Sample and treatment	Total solids	pH	Specific conductance in $\mu$ mho at 25 C	% Nitrogen, dry weight basis		
				Total	Non-protein <sup>a</sup>	Protein
None	7.60	6.0	8,330	.698	.462	.236
Sirup I						
Deproteinized <sup>b</sup>	7.45	4.5	8,540	.400	.400	.0
IR 252 (H <sup>+</sup> )	7.00	1.4 - 1.5	18,900 - 23,300	.271	.271	.0
IRA 93 (OH <sup>-</sup> )	6.10	5.6 - 9.3	54 - 198	.190	.190	.0
Sirup II						
IR 252 (H <sup>+</sup> )	6.94	1.5 - 1.6	15,300 - 16,700	.557	.327	.230
IRA 93 (OH <sup>-</sup> )	6.00	6.6 - 9.1	19 - 61	.293	.193	.100
Deproteinized <sup>c</sup>	5.90	4.5	...	.185	.185	.00

<sup>a</sup>Total N minus protein N.<sup>b</sup>3.50 g presoaked granular bentonite (1:10 H<sub>2</sub>O)/g protein at pH 4.5.<sup>c</sup>2.50 g presoaked granular bentonite (1:10 H<sub>2</sub>O)/g protein at pH 4.5.

TABLE 3. Effect of pH on nitrogen removal from whey permeates by IRA 93 resin.

Sample treatment of Sirup III	% Total solids	pH	Specific conductance $\mu$ mho at 25 C	% Nitrogen, dry weight basis		
				Total	Non-protein <sup>a</sup>	Protein
None	7.30	6.0	7,830	.698	.462	.236
Deproteinized <sup>b</sup>	7.05	4.5	8,200	.354	.354	.0
IR 252	6.80	1.40 - 1.42	16,700 - 20,900	.218	.218	.0
IRA 93						
First pass <sup>c</sup>						
K	5.50	9.7 - 9.3	84 - 195	.217	.217	.0
P	6.00	9.8 - 9.3	145 - 149	.117	.117	.0
L	6.15	9.2 - 7.7	61 - 192	.276	.276	.0
M	5.80	6.0 - 4.8	55 - 151	.172	.156	.016
Second pass						
Blend of K, P and L	5.60	9.7 - 9.4	124 - 171	.218	.218	.0

<sup>a</sup>Total N minus protein N.

<sup>b</sup>3.50 presoaked granular bentonite/g protein at pH 4.5 plus 1% wt/vol Norit.

<sup>c</sup>Volume ratio L = 1.00, P = .55, K = 1.18, M = .52.

TABLE 4. Analysis of sirups.

Sirup	pH	Dry weight basis		
		% (N × 6.38)	% Ash	% Carbo- hydrated
I	5.0	1.21	.132 <sup>a</sup>	98.66
II	5.1	1.18	.184 <sup>a</sup>	98.64
III	5.3	1.39	.230 <sup>a</sup>	98.38
V <sup>b</sup>	5.3	2.69	8.31 <sup>c</sup>	89.00
			8.68 <sup>a</sup>	88.63

<sup>a</sup>As chloride (sulfated ash × .85).

<sup>b</sup>Deproteinized with 13.0 g bentonite/g protein at pH 6.0 in an 11.7% total solids permeate.

<sup>c</sup>Conventional ash.

<sup>d</sup>Determined by difference.

Comparison of the three purification procedures (Tables 2 and 3) showed that sirups I, II, and III had comparable NPN, but sirup I had the lowest ash (Table 4). The analysis of deproteinized permeates (V) also is given. To support our findings that complete nitrogen and ash removal from sugar is difficult, McGlasson and Boyd (14) reported that removal of protein from whey by heat precipitation and addition of CaCl<sub>2</sub> followed by passage through ion exchange resins three times, resulted in lactose with 2.2% (N × 6.38) and .18% ash (dry weight basis).

#### Color Stability

An approximation of the color stability of corn sirups can be obtained by measuring the color gain when a sample is heated 1 h at 100 C. When the purified sirups obtained from permeate were heated to 100 C for 1 h, their color changed from water-white to light-tan,

measured spectrophotometrically (Table 5), probably because of the caramelization reaction between N and carbohydrate. No increase in color was observed when HL sirups were similarly heated.

#### Stability of Sirups

At 60% TS, sirups were variably stable if heated at 70 C in bottles and then sealed (Table 6). At comparable TS, sirups isolated from permeate were as stable as or slightly more so than sirups made from hydrolyzed USP lactose. Above 60% TS, all sirups crystallized. Mold formed within 2 to 6 wk in unheated sirups at 60% TS.

#### Sweetness of Sirups

At comparable TS, the sirups were not as sweet as sucrose in the 20 to 40% TS range (Table 7), and the sweetness of the sirups relative to sucrose increased with increasing TS.

TABLE 5. Optical density increase in sirups from 600 mμ to 450 mμ following heating for 1 h in a boiling water bath.

	% Non- protein nitrogen	Optical density increase/ cm
Sirup I	1.21	.0680
Sirup II	1.18	.0627
90% Hydrolyzed lactose sirup	.0	.0

TABLE 6. Stability of sirups stored at room temperature.

Sirup prepared from permeates	% Total solids	Weeks to crystallize (C) or mold (M)	
		Un-heated	Heated at 70 C in sealed bottle
I	60		
II	60	2 - 6 (M)	>40
III	60	6 (M)	18 (C)
	62.7	3 - 4 (M)	>22, 15 (C)
	65.6	3 - 4 (C)	7 (C)
		2 (C)	4 (C)
90% Hydrolyzed lactose	60.3	4 (C)	4 (C), >22
	62.7	1 - 2 (C)	...
	66.2	1 (C)	...

Blending of deproteinized, but not demineralized (Table 7), sirup V slightly decreased the sweetness of the mixture, contributed to more off flavors and slight saltiness, and is not recommended to produce an acceptable sirup. Treating the sirups with 2% Norit carbon decreased the number of off flavors in both sirups. The off flavors, described as bitter, caramel, or medicinal, were slight. Test sirups of 60% TS were stored at 5 C. They then were heated to dissolve crystals, diluted with distilled

water, and evaluated. Sucrose sirups were freshly prepared from cane sugar.

#### CONCLUSIONS

Whey from the manufacture of cheese from lactase-hydrolyzed whole milk yields whey permeates of lowered nitrogen content but rich in carbohydrates and salts. Utilization of these permeates is important to the economics of whey production. This work demonstrates

TABLE 7. Sweetness equivalents of sirups.

Sirup	% Total solids	Sweetness, % sucrose equivalent	No. of judges indicating some type of off flavor
I			
90% Hydrolyzed lactose control	40.0	35.7 <sup>ab</sup>	7
II <sup>d</sup>	40.0	34.7 <sup>ab</sup>	8
92.5% II <sup>d</sup> + 7.5% V	40.0	35.9 <sup>a</sup>	3
85% II <sup>d</sup> + 15% V	40.0	32.6 <sup>b</sup>	4 + 2 salty
III <sup>d</sup>	40.0	33.7 <sup>b</sup>	7 + 3 salty
	40.0	33.7 <sup>b</sup>	4
90% Hydrolyzed <sup>d</sup> lactose control	20.0	15.1 <sup>c</sup>	1
	40.0	32.0 <sup>b</sup>	2
	20.0	14.6 <sup>c</sup>	2

<sup>a</sup>Significantly different at 5% from 30% total solids (TS) sucrose.

<sup>b</sup>Significantly different at 5% from 40% TS sucrose.

<sup>c</sup>Significantly different at 1% from 20% TS sucrose.

<sup>d</sup>Treated with 2% Norit.

that clear, colorless sirups of low ash and nitrogen can be isolated from HL whey permeates by treatment with bentonite to remove residual protein and by ion exchange resins to remove salts. In sweetness and stability these sirups are equivalent to or more than those produced by the hydrolysis of crystalline lactose. To increase their stability, the sirups, at high solids, might be used for blending with corn or sucrose sirups, providing the economics are right. Likewise, 75% hydrolysis of lactose increases the stability of concentrated sirups (7). Less purified sirups isolated from acid whey treated with  $\beta$ -galactosidase (21) have been suggested for use in yoghurts, puddings, nutrient fruit juice, and imitation maple sirup.

The bentonite used to adsorb protein settled readily and was recovered easily by siphoning off the supernatant. The bentonite containing high quality protein should be most suitable for feed rations. Feed rations containing 5 to 10% bentonite increased fat yield and milk yield significantly in dairy cows (19). Bentonite, in addition to removing all the protein, removed about 13% of nonprotein nitrogen and presumably adsorbed the riboflavin, yielding a colorless whey permeate.

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